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Interactions Between β-Herpesviruses and Human Immunodeficiency Virus In Vivo: Evidence for Increased Human Immunodeficiency Viral Load in the Presence of Human Herpesvirus 6

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In vitro, β-herpesviruses can stimulate or inhibit HIV replication under particular circumstances. In order to investigate the effects of β-herpesvirus infection on HIV replication and vice versa at an organ level, we determined the quantitative relationships between cytomegalovirus (CMV), human herpesviruses (HHV) 6 and 7, and HIV-1 proviral DNA using quantitative competitive PCR methods in 141 organs collected at autopsy from 11 AIDS patients. The presence of HHV-6 DNA in an organ was significantly associated with elevated HIV-1 proviral DNA (difference in HIV median loads, 1.3 $\log_{10}$ genomes; $P = 0.004$). Consistent with this, there was a trend for the presence of HIV-1 proviral DNA to be associated with an elevated HHV-6 load (0.44 $\log_{10}$ difference; $P = 0.07$). In contrast, there were no significant differences between viral loads in the combinations of either CMV or HHV-7 with HIV-1 proviral DNA load. Pairwise combinations of the β-herpesviruses revealed that the quantity of HHV-7 was increased in the presence of HHV-6 (difference in median loads, 1.3 $\log_{10}$; $P = 0.001$) and the quantity of HHV-6 was increased in the presence of HHV-7 (difference in median loads, 0.7 $\log_{10}$; $P = 0.002$). These results demonstrate that the presence of HHV-6 in an organ is significantly associated with an elevated HIV-1 proviral load and have implications for understanding HIV pathogenesis in the human host and the role that β-herpesviruses, especially HHV-6, might play as cofactors in the HIV disease process. ***J. Med. Virol. 57:278–282, 1999.***

KEY WORDS: cofactor; postmortem; QCPCR

INTRODUCTION

Human immunodeficiency virus (HIV) causes acquired immune deficiency syndrome (AIDS) by inducing progressive damage to cell-mediated immune responses. By the time patients with HIV disease are profoundly immunocompromised, they frequently suffer from a variety of opportunistic virus infections and tumors [Hoover et al., 1993; Moore and Chaisson, 1996]. These virus infections may cause disease in their own right, such as cytomegalovirus (CMV) retinitis, varicella zoster virus acute retinal necrosis, and herpes simplex virus esophagitis [Gallant et al., 1992; Pertel et al., 1992; Glesby et al, 1995]. In addition, it is theoretically possible that any of the herpesviruses may interact with HIV to accelerate the rate at which it causes immune deficiency [reviewed in Griffiths, 1992, 1998; Lusso and Gallo, 1995].

The β-herpesviruses, comprising CMV, human herpesvirus (HHV) type 6, and HHV-7, all have the potential to modulate HIV disease pathogenesis by a variety of mechanisms, summarized in Table I. Numerous in vitro studies have shown that CMV [Gendelman et al., 1986; McKeating et al., 1990] and HHV-6 [Ensoli et al., 1989; Lusso et al., 1991] can upregulate the replication of HIV-1. However, for CMV [Koval et al., 1991, 1995] and HHV-6 [Carrington et al., 1990; Levy et al., 1990], both stimulatory and inhibitory effects have been observed depending on cell type and type of in vitro assay used. In addition, HHV-7, the most recently character-

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TABLE I. Interactions Between β-Herpesviruses and HIV Studied In Vitro

Effect of interaction	Proximity of HIV/herpesvirus	Mechanism of interaction	Virus studies in vitro[a]
Activate	Same cells	Transactivation	CMV↑ HHV-6↑
HIV proviral	Neighboring cells	Cytokine release	CMV↑ HHV-6→
DNA		Antigen presentation	CMV↑
Alter	Same cells	Pseudotype formation	CMV↑
tropism	Neighboring cells	Induce HIV receptor/coreceptor	CMV↑ HHV-6↑ HHV-7↓
HIV		Induce alternative HIV receptor	CMV↑

[a]Arrows refer to effect of β-herpesvirus on HIV replication: ↑ = upregulation; → = no effect; ↓ = downregulation.

ized β-herpesvirus, utilizes CD4 as a cellular receptor and has been shown to compete with HIV in vitro for target CD4 cells [Lusso et al., 1994; Crowley et al., 1996]. Thus, while there has been much interest and speculation on the possible significance for patients of these in vitro findings, there is a paucity of substantive data to show that HIV replication is affected in vivo by β-herpesvirus infections. Some [Webster et al., 1989; Detels et al., 1994; Sabin et al., 1995], but not all [Rabkin et al., 1993], epidemiologic studies have implicated CMV as a cofactor for HIV disease progression, while CMV and HHV-6 have been implicated on the basis of widespread evidence of infection in organs of patients dying with AIDS [Nelson et al., 1988; Corbellino et al., 1993; Pillay et al., 1993; Clark et al., 1996]. Since it is now firmly established that the progression of HIV disease is strongly linked to HIV-1 viral load [Mellors et al., 1996], we addressed the possibility that the β-herpesviruses may facilitate replication of HIV-1 in vivo by analyzing the relationships between the quantity of HIV-1 proviral DNA in multiple organs obtained at autopsy from AIDS patients and the presence of CMV, HHV-6, and HHV-7 in the same organs.

MATERIALS AND METHODS

Patients

Eleven unselected HIV-1–seropositive individuals who died with AIDS-defining illnesses were subjected to full autopsy and samples of up to 14 organs obtained (lymph node, spleen, brain, lung, heart, kidney, adrenal, esophagus, duodenum, colon, pancreas, liver, stomach, and salivary gland). The total number of organs derived from these patients was 141 (median, 14 organs per patient; range, 9–14). The CD4 counts of the patients at death ranged from 0 to 20 cells per cubic millimeter (median, 10) and all but two patients had been prescribed zidovudine for their HIV infection for between 8 and 47 months (mean duration of therapy, 27.25 ± SD 13.5 months).

Extraction of DNA From Tissues

A 125-mm^3 (approximately 5 mm × 5 mm × 5 mm) block of frozen tissue was finely dissected and washed three times with sterile PBS (1 ml) and centrifuged in a microfuge between washes to remove residual blood. DNA was extracted from the tissue using the Promega Wizard DNA preparation kit according to the manufacturer's instructions. DNA was resuspended in water and 1 μg of DNA (equivalent to approximately 1.5×10^5 diploid cells) used for all subsequent analysis.

Qualitative and Quantitative PCR Detection of CMV, HHV-6, HHV-7, and HIV-1

The qualitative PCR methods for the detection of CMV, HHV-6, HHV-7, and HIV-1 and quantitative competitive PCR (QCPCR) methods used to quantify the viral burden of each virus have been previously described [Fox et al., 1992; Atkins et al., 1996; Clark et al., 1996; Kidd et al., 1997]. The sensitivity of the methods was comparable with the ultimate sensitivity of detection of CMV being 5 genome equivalents per μg DNA, whilst the HHV-6, HHV-7, and HIV-1 proviral DNA nested QCPCR assays were capable of detecting 2 genome equivalents per μg DNA. Differentiation of HHV-6 strains A or B was performed by Ava II digestion of the HHV-6 amplicons, as described elsewhere [Kidd et al., 1998].

Statistical Analysis

Median viral loads were compared using the Mann-Whitney U-test. Relationships between the presence of one virus as detected by PCR and the quantity of another virus were investigated and differences in viral loads between the two groups assessed using the Mann-Whitney U-test. All organs (n = 141) from the 11 AIDS patients were included in these analyses without associating specific organs with different patients.

RESULTS

Interactions Between β-Herpesviruses in Organs From AIDS Patients

CMV, HHV-6 (variant B), HHV-7, and HIV-1 proviral DNA were present in 67.4%, 71.6%, 65.7%, and 64.3%, respectively, of organs obtained from patients who died with AIDS. No organ had HHV-6 variant A. The influence of the presence of one β-herpesvirus on the quantity of another β-herpesvirus was first investigated (Fig. 1). There was a bidirectional interaction between HHV-6 and HHV-7 such that the presence of HHV-6 resulted in a higher viral load of HHV-7 and vice versa. The difference in median HHV-7 viral load between organs with or without HHV-6 DNA was 1.3 $\log_{10}$, while the difference in median HHV-6 viral load between organs with or without HHV-7 DNA was 0.7 $\log_{10}$. There were no statistically significant differences

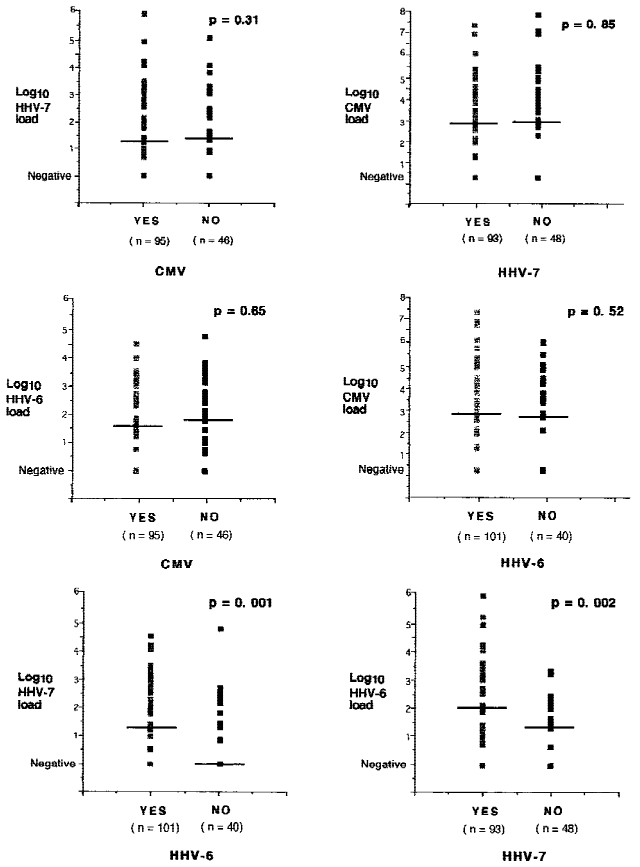

Fig. 1. Influence of the presence of one β-herpesvirus on the quantity of a second β-herpesvirus in the organs of AIDS patients. A total of 141 organs from 11 AIDS patients are included in the analysis. The horizontal line within each group indicates the median and the significance of differences between groups is shown (Mann-Whitney U-test). The negative symbol represents multiple samples in all cases.

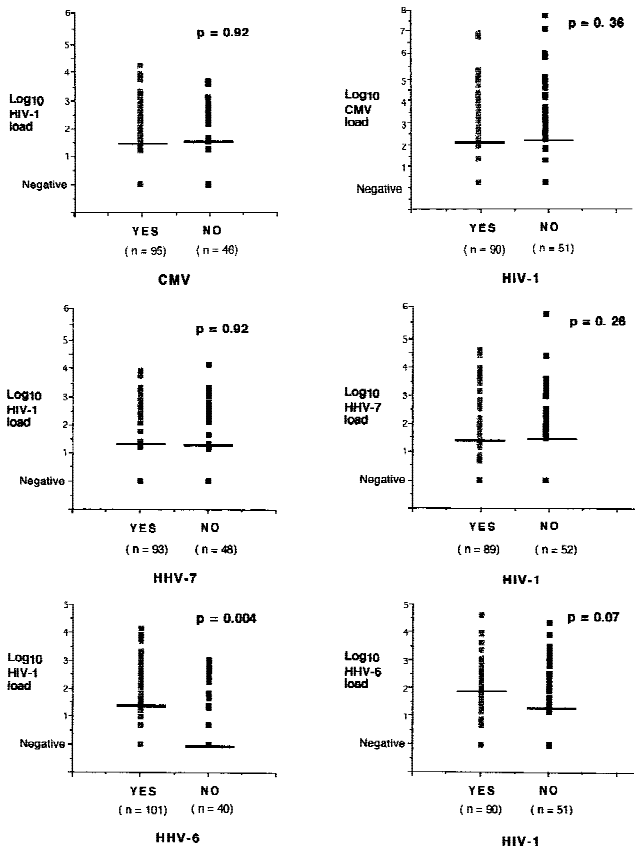

Fig. 2. Influence of the presence of β-herpesvirus DNA on the proviral load of HIV-1 and the reciprocal presence of HIV-1 proviral DNA on β-herpesvirus DNA load. A total of 141 organs from 11 patients are included in the analysis. The median of each group is shown as a horizontal line together with significance of differences (Mann-Whitney U-test). The presence of HHV-6 DNA was associated with a significantly elevated HIV-1 proviral DNA load.

between viral loads in the other combinations of β-herpesviruses, i.e., CMV and HHV-6 or CMV and HHV-7.

Interactions Between β-Herpesviruses and HIV-1 in Organs

Similar analyses to those shown in Figure 1 were used to ascertain the influence of β-herpesvirus detection on HIV-1 proviral load and vice versa (Fig. 2). There was a significant elevation of HIV-1 proviral DNA in organs that contained HHV-6 DNA ($P = 0.004$) with a median proviral load 1.3 $\log_{10}$ higher than organs without HHV-6 DNA. In addition, there was a trend, which approached statistical significance, for the presence of HIV-1 proviral DNA to be associated with an elevated HHV-6 load (0.44 $\log_{10}$ difference; $P = 0.07$). Similar effects were not observed for the CMV–HIV-1 or HHV-7–HIV-1 combinations.

The results of median load measurements are given in Table II, together with statistical assessment of any differences observed. It is apparent that only pairwise comparisons of viruses that included HHV-6 were associated with significant increases in viral loads of any of the viruses measured. Multiple linear regression analyses were also carried out on the data with similar results to those obtained by the nonparametric methods shown in Table II (data not shown).

DISCUSSION

The results of this investigation provide evidence of distinct differences in the in vivo relationships between each of the three β-herpesviruses and HIV. The viral burden of HIV (as judged by proviral DNA) was significantly increased in organs coinfected with HHV-6. This effect is biologically plausible as the highest viral loads for HIV-1 are present in the lymphoid tissues and both HHV-6 and HIV-1 are tropic for T-cells [Lusso et al., 1995]. Previous qualitative PCR data have shown that a high proportion of organs from AIDS patients harbor HHV-6 DNA [Corbellino et al., 1993] and that disseminated active HHV-6 infection has been observed via immunohistochemistry of paraffin-embedded tissues from AIDS patients [Knox and Carrigan, 1994, 1996]. Our group has previously shown that HHV-6 viral load is elevated in organs from AIDS patients compared with HIV-negative controls [Clark et al., 1996]. Thus, on the basis of the results reported here, in vitro data

TABLE II. Association Between the Presence of Each of Four Viruses and the Quantity of DNA From the Remaining Three Viruses in Autopsy Tissues

Virus DNA quantified	Median viral load (log_{10} genomes/μg) in the presence of each virus detected qualitatively											
	CMV			HHV-6			HHV-7			HIV		
	Yes	No	*P*	Yes	No	*P*	Yes	No	*P*	Yes	No	*P*
CMV				2.87	2.86	0.52	2.79	3.10	0.78	2.79	3.05	0.36
HHV-6	1.69	1.93	0.65				2.00	1.30	0.002	1.85	1.41	0.07
HHV-7	1.30	1.30	0.42	1.30	0	0.001				1.30	1.36	0.20
HIV	1.30	1.30	0.52	1.30	0	0.004	1.30	1.30	0.47			

showing that HHV-6 is capable of upregulating HIV replication appear to translate into a true in vivo effect.

In contrast to the positive bidirectional association between HHV-6 and HIV-1 proviral loads, neither CMV nor HHV-7 was shown to produce the same elevation in HIV-1 load. Hence, the results do not support a cofactor role for CMV or HHV-7 at the organ level at this late stage of HIV disease, despite the fact that CMV and HIV-1 can be found in the same tissues [Nelson et al., 1988; Webster et al., 1995]; CMV encodes a chemokine receptor that can act as a cofactor for HIV-1 entry [Pieskoff et al., 1997] and HHV-7 and HIV-1 are both tropic for CD4 T-cells [Lusso et al., 1994; Crowley et al., 1996]. The finding that CMV burden in organs was similar, irrespective of the presence of HIV, argues against a mutual positive interaction between these viruses in vivo and illustrates that in vitro results (summarized in Table I) cannot be extrapolated directly to the human host. Nevertheless, these autopsy findings do not exclude the possibility that CMV and HIV may interact at earlier stages, as has been suggested by some, but not all, studies [Rabkin et al., 1993; Detels et al., 1994; Sabin et al., 1995]. We can exclude the possibility that the current study lacks statistical power to detect a cofactor effect of CMV because such an effect was seen for HHV-6. Since the data show that the median viral load of CMV in organs was significantly greater than that of either HHV-6 ($P = 0.0001$) or HHV-7 ($P = 0.0001$), the major clinical effect of CMV at this late stage of HIV disease may be to cause disease in its own right. Such an observation is consistent with the fact that clinically recognized CMV disease, particularly retinitis, has been a major clinical problem for AIDS patients [Gallant et al., 1992; Pertel et al., 1992] and that patients with high CMV viral loads have significantly increased rates of death [Bowen et al., 1996; Spector et al., 1998]. Since there was no difference in the median viral load of HHV-7 within organs of AIDS patients with or without HIV-1 proviral DNA or vice versa, the data do not support the concept that competition between HHV-7 and HIV-1 for the CD4 receptor has a major effect on reducing HIV-1 or HHV-7 load in vivo.

Multiple mechanisms by which HHV-6 could stimulate HIV replication have been described, including upregulation of CD4 expression in CD8 cells and natural killer cells [Lusso et al., 1991, 1993] and transactivation by HHV-6 genes [Ensoli et al., 1989; Lusso et al., 1989]. Although these latest results do not directly implicate any particular mechanism, we suggest that comparison of the results for HHV-6 and HHV-7 may help to prioritize investigations into likely potential interactions. Thus, the genomes of HHV-6 and HHV-7 are highly homologous [Gompels et al., 1995; Nicholas, 1996] so that genes present in HHV-6 but absent from HHV-7 are prime candidates for further study. Specifically, the *rep* gene from adeno-associated viruses is present in HHV-6 [Thomson et al., 1991] but not in HHV-7 [Nicholas, 1996] and has been shown to transactivate HIV [Thomson et al., 1994]. Alternatively, differences in cell tropism between HHV-6 and HHV-7 could explain their differential effects on HIV; in situ techniques could be employed in autopsy tissues to identify whether HHV-6:HIV coinfection occurs or whether mechanisms requiring infection of neighboring cells by each virus are more plausible (see Table I). We plan to conduct such studies and extend the tissues investigated to include those taken as biopsies during life. Indeed, one study has reported active HHV-6 infection in lymph node biopsies taken relatively early in the course of HIV disease [Knox and Carrigan, 1996], implying that such an effect may be operational over long periods of time. Since HHV-6 infection is almost universal in the population, the effects of HHV-6 on HIV-1 replication could modulate the pathogenesis of HIV-1 in the majority of HIV-1–seropositive individuals.

The complexity of studying potential cofactors for HIV disease progression in vivo is aptly demonstrated when the effects of each β-herpesvirus on each other's replication are investigated. The data show a highly significant correlation between HHV-6 and HHV-7 viral loads in the organs from AIDS patients, implying a symbiotic relationship between these two infections. Whether this represents the local environment of an organ providing a milieu of cytokines and cellular activation conducive to replication or is a direct effect of each virus on the other cannot be answered from this study. However, in vitro data support the ability of HHV-6 replication to reactivate HHV-7 [Wyatt and Frenkel, 1992; Katsafanas et al., 1996].

In summary, evidence is presented that HHV-6 can facilitate HIV replication at an organ-specific level and that these effects may reflect a complex balance between the interactions of multiple β-herpesviruses. Knowledge of the natural history and pathogenesis of these viruses is important for understanding the progression of HIV disease and its symptomatology and the results suggest new approaches to managing HIV

disease which, while speculative and provocative, can be tested in controlled clinical trials of antiviral agents with activity against one or more β-herpesviruses.

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